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Growth Factors, Cytokines, Cell Cycle Molecules

The Role of Macrophage Migration Inhibitory Factor in the Cascade of Events Leading to Reperfusion-Induced Inflammatory Injury and Lethality

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Ischemia and reperfusion (I/R) injury is associated with a systemic inflammatory response, characterized by intense tumor necrosis factor (TNF)-α production and TNF-α-dependent tissue injury. Macrophage migration inhibitory factor (MIF) is a potent proinflammatory cytokine that may induce TNF-α release and play an important role in innate immune and inflammatory responses. The aim of this work was to assess whether MIF was involved the inflammatory cascade and injury that follows intestinal I/R. To this end, wild-type (WT) and MIF-deficient ($MIF^{-/-}$) mice underwent 60 minutes of ischemia followed by 60 minutes of reperfusion, after which they were culled for the assessment of inflammatory parameters. I/R was accompanied by an increase in circulating levels of MIF and an increase of vascular permeability, hemorrhage, and production of TNF-α in the intestine and lungs. The latter parameters were markedly suppressed in reperfused $MIF^{-/-}$ mice, and this was associated with decreased lethality (80% in WT versus 20% in $MIF^{-/-}$ mice). Interestingly, the reperfusion-associated neutrophil accumulation in the intestine and lungs was similar in WT and $MIF^{-/-}$ mice. Leukocytes isolated from lungs of $MIF^{-/-}$ mice were less activated, as assessed by their response to zymosan in a luminol-enhanced chemiluminescence assay. In conclusion, our results suggest that MIF plays an important role in the cascade of events leading to TNF-α production and reperfusion-induced tissue injury and lethality in mice. ***(Am J Pathol 2007, 171:1887–1893; DOI: 10.2353/ajpath.2007.060642)***

Macrophage migration inhibitory factor (MIF) was originally described as a cytokine released from activated T cells that inhibited the random migration of macrophages.[1,2] More recently, several other important functions of MIF in innate and adaptive immune responses and in inflammation have been described.[3] MIF may be released by macrophages,[4] T cells,[5] neutrophils,[6] vascular endothelial cells,[7] and by the anterior pituitary gland.[8] An interesting characteristic of MIF, as opposed to other cytokines, is that it is induced rather than inhibited by low doses of glucocorticoid hormones.[9] Studies using MIF-deficient ($MIF^{-/-}$) mice and anti-MIF antibodies have shown that MIF may modulate the production or expression of several proinflammatory molecules, including cytokines, nitric oxide, and in the activation of the cyclooxygenase pathway.[3] By controlling immune and inflammatory responses, MIF is thought to play an important role in the pathophysiology of septic shock,[8,10,11] rheumatoid arthritis,[12–14] and inflammatory lung diseases.[15–17] The role of MIF in mediating the influx of leukocytes, production of cytokines, and tissue injury in reperfusion syndromes has not been described to date.

The reperfusion of the ischemic superior mesenteric artery of mice is accompanied by a significant local (intestine), remote (lungs), and systemic inflammatory response.[18,19] Studies from our and other groups have clearly established a central role for tumor necrosis factor (TNF)-α in mediating tissue injury and lethality in this model.[19–22] Because MIF can be rapidly released from intracellular stocks in leukocytes[4,6] and has the capacity to modulate the production of TNF-α,[5,10,23] we investigated the contribution of MIF to the injury and lethality that occurs after reperfusion of the ischemic mesenteric artery in mice.

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Materials and Methods

Animals

Breeding pairs of SV129 MIF-deficient ($MIF^{-/-}$) mice were kindly provided by Dr. John R. David, Harvard School of Public Health, Boston, MA, and were generated as previously reported.[10] $MIF^{-/-}$ and wild-type (WT) male SV129 mice (10 to 12 weeks of age) were bred at and obtained from Instituto Gonçalo Muniz, Bahia, Brazil, and housed under standard conditions with free access to commercial chow and water. MIF deficiency was confirmed by polymerase chain reaction, as previously described (data not shown).[10] All procedures described here had prior approval of the local animal ethics committee.

Ischemia and Reperfusion (I/R)

Mice were anesthetized with urethane (1400 mg/kg, i.p.) and laparotomy was performed. The superior mesenteric artery was isolated, and ischemia was induced by totally occluding the superior mesenteric artery for 60 minutes. For measuring percentage of surviving mice, reperfusion was re-established, and mice were monitored for the indicated time periods. For the other parameters, reperfusion was allowed to occur for 60 minutes (I60R60), after which mice were sacrificed. This time of reperfusion (60 minutes) was chosen based on the presence of significant tissue injury without unduly high mortality rates in the present strain of mice. Sham-operated animals were used as controls.

Evaluation of Changes in Vascular Permeability

The extravasation of Evans blue dye into the tissue was used as an index of increased vascular permeability, as previously described.[24] Evans blue (20 mg/kg) was administered intravenously (1 ml/kg) via a tail vein 5 minutes before reperfusion of the ischemic artery. Sixty minutes after reperfusion, a segment of the duodenum (at ~5 cm) was cut open and allowed to dry in a Petri dish for 24 hours at 37°C. The dry weight of the tissue was calculated and Evans blue extracted using 1 ml of formamide (24 hours at room temperature). The amount of Evans blue in the tissue was obtained by comparing the extracted absorbance with that of a standard Evans blue curve read at 620 nm in an enzyme-linked immunosorbent assay (ELISA) plate reader. Results are presented as the amount of Evans blue per μg per 100 mg of tissue. The right ventricle was flushed with 10 ml of phosphate-buffered saline (PBS) to wash the intravascular Evans blue in the lungs. The left lung was then excised and used for Evans blue extraction. The right lung was used for the determination of myeloperoxidase as described below.

Myeloperoxidase Concentrations

The extent of neutrophil accumulation in the intestine and right lung tissue was measured by assaying myeloperoxidase activity, as previously described.[19] Briefly, a portion of duodenum and the flushed right lungs of animals that had undergone I/R injury were removed and snap-frozen in liquid nitrogen. On thawing and processing, the tissue was assayed for myeloperoxidase activity by measuring the change in optical density (O.D.) at 450 nm using tetramethylbenzidine. Results were expressed as the neutrophil index that denotes activity of myeloperoxidase related with casein-elicited murine peritoneal neutrophils processed in the same way.

Measurement of Hemoglobin Concentrations

The determination of hemoglobin concentrations in tissue was used as an index of tissue hemorrhage. After washing the intestines to remove excess blood, a sample of ~100 mg of duodenum was removed and homogenized in Drabkin's color reagent according to instructions of the manufacturer (Analisa, Belo Horizonte, Brazil). The suspension was centrifuged for 15 minutes at 3000 $\times$ *g* and filtered using 0.2-μm filters. The resulting solution was read using an ELISA plate reader at 520 nm and compared against a standard curve of hemoglobin.

Measurement of Cytokine in Serum, Intestine, and Lung

The concentration of murine TNF-α and keratinocyte-derived chemokine (KC) in samples was measured in serum and tissue of animals using commercially available antibodies and according to the procedures supplied by the manufacturer (R&D Systems, Minneapolis, MN). ELISA kits to determine murine MIF are not available, and a human MIF ELISA (R&D Systems) was used. Serum was obtained from coagulated blood (15 minutes at 37°C, then 30 minutes a 4°C) and stored at −20°C until further analysis. Serum samples were analyzed at a 1:3 dilution in PBS. One hundred mg of duodenum or lung of sham-operated and reperfused animals were homogenized in 1 ml of PBS (0.4 mol/L NaCl and 10 mmol/L $NaPO_4$) containing anti-proteases (0.1 mmol/L phenylmethyl sulfonyl fluoride, 0.1 mmol/L benzethonium chloride, 10 mmol/L ethylenediaminetetraacetic acid, and 20 KI aprotinin A) and 0.05% Tween 20. The samples were then centrifuged for 10 minutes at 3000 $\times$ *g* and the supernatant immediately used for ELISA assays at a 1:3 dilution in PBS.

Histopathology

Organs (duodenum and lungs) were removed from anesthetized mice at indicated time points and immediately fixed in formalin for 24 hours, and tissue fragments were embedded in paraffin. Tissue sections (4 μm thick) were stained with hematoxylin and eosin (H&E).

Lung Leukocyte Extraction with Collagenase and Evaluation of Cell Respiratory Burst Induced by Unopsonized Zymosan Using a Luminol-Enhanced Chemiluminescence Assay

Lung cell suspensions were prepared by perfusing heparinized ice-cold 0.9% saline solution through the heart of mice in all groups at the end of each experiment. Once lungs appeared clean from blood, they were removed and sectioned in ice-cold RPMI 1640 medium (RPMI 1640 medium without neutral red; Sigma Chemicals, St. Louis, MO) using sterile razor blades. Dissected lung tissue was then incubated in collagenase IX (0.7 mg/ml, Sigma Chemicals) at 37°C for 30 minutes.[25] Digested lung tissue was gently disrupted by passages through a 70-μm nylon cell strainer, and the residual red blood cells removed by osmotic lysis. The resultant cell suspension, composed of 70 to 80% polymorphonuclear cells, of different lungs was pooled and washed in ice-cold RPMI 1640 and counted. This cell preparation was used for respiratory burst evaluation by luminol-enhanced chemiluminescence assay induced by unopsonized zymosan.

Luminol-enhanced and zymosan-induced chemiluminescence was measured to assess cell respiratory burst. After cell counting, a pool of cells from each group (sham and I/R from WT and MIF$^{-/-}$ mice), resuspended in colorless RPMI 1640, were plated in triplicate at 3×10^5 cells/well in a Luminunc 96-well microwell plate, maxisorp, white, flat bottom (Nunc Brand Products, Rochester, NY). Luminol (5×10^{-5} mol/L) and zymosan (20 particles/cell) (Sigma Chemicals) were added in each well. The zymosan-elicited luminescence was then recorded by a luminometer (LumiCount; Packard, Meriden, CT) for 120 minutes at 37°C (60 cycles of measurement with intervals of 2 minutes). Chemiluminescence emission of each well was expressed as relative luminescence units. The area under the curve was calculated from the curves generated by the assay to evaluate the different profiles of luminol-enhanced chemiluminescence from each group.

Statistical Analysis

Results are shown as the mean ± SEM. Percent inhibition was calculated by subtracting the background levels of Evans blue extravasation or myeloperoxidase (obtained in sham-operated animals) from control and treated animals. Differences were evaluated by using analysis of variance followed by Student-Newman-Keuls post hoc analysis. Results with a $P < 0.05$ were considered significant. For survival curves, differences between groups at different time points were compared using Fisher's exact test and considered significant when $P < 0.05$.

Results

Level of MIF in the Serum after I/R Injury

Initial experiments were designed to assess whether MIF could be measured in the serum of animals submitted to I/R injury. After 60 minutes of ischemia followed by 60 minutes of reperfusion, the amount of MIF immunoreactivity present in serum was significantly greater in reperfused than sham-operated mice (Figure 1).

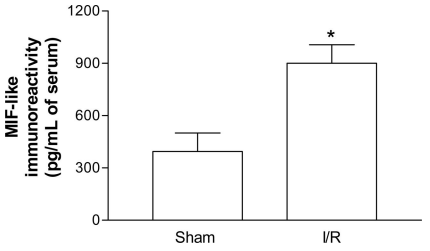

Figure 1. Concentration of MIF in serum of mice undergoing intestinal I/R injury. The concentration of MIF was determined by ELISA in serum of sham-operated mice (Sham) and mice undergoing 60 minutes of ischemia and 60 minutes of reperfusion (I/R) of the superior mesenteric artery. Results are shown as pg of MIF per ml of serum and are mean ± SEM of five animals. *$P < 0.01$ when compared to sham-operated animals.

Measurement of TNF-α in Absence of MIF after I/R Injury

Because MIF has been shown to modulate TNF-α release in other inflammatory reactions,[5,10,23] the next step was to evaluate the effects of MIF deficiency on the levels of TNF-α in the intestine, lungs, and serum of reperfused mice. There was no detectable expression of TNF-α in the intestine and serum and low level of expression of TNF-α in the lungs of sham-operated WT or MIF$^{-/-}$ mice (Figure 2 and Table 1). In animals submitted to I/R, the levels of TNF-α rose markedly in all three compartments. This was not observed in MIF$^{-/-}$ mice. Indeed, in the latter animals, TNF-α was not detectable in intestine and serum (Figure 2) and did not rise above baseline levels in the lungs (Table 1).

Measure of Inflammatory Parameters in Absence of MIF

The reperfusion of ischemic mice also induced an increase in other inflammatory parameters, including a local increase of vascular permeability, hemorrhage, and neutrophil influx (Figures 3 and 4). There was also significant remote injury to the lung, as seen by the increase in vascular permeability and neutrophil accumulation (Table 1 and Figure 4). Consistently with the role of TNF-α in mediating tissue injury in this model[19,20] and with the suppression of TNF-α production in MIF$^{-/-}$ mice, there was a marked inhibition of vascular permeability increase and hemorrhage in the intestine of MIF$^{-/-}$ mice (Figure 3, A and B). There was prevention of the increase of vascular permeability in the lungs of reperfused MIF$^{-/-}$ mice (Table 1). Despite the suppression of TNF-α production, vascular permeability changes, and hemorrhage, the neutrophil influx induced by the reperfusion process was similar in WT and MIF$^{-/-}$ mice (Figure 3C and Table 1). Interestingly and consistently with a lack of effect of the MIF$^{-/-}$ phenotype on neutrophil influx, the intestinal levels of the neutrophil-active chemokine KC were similar in

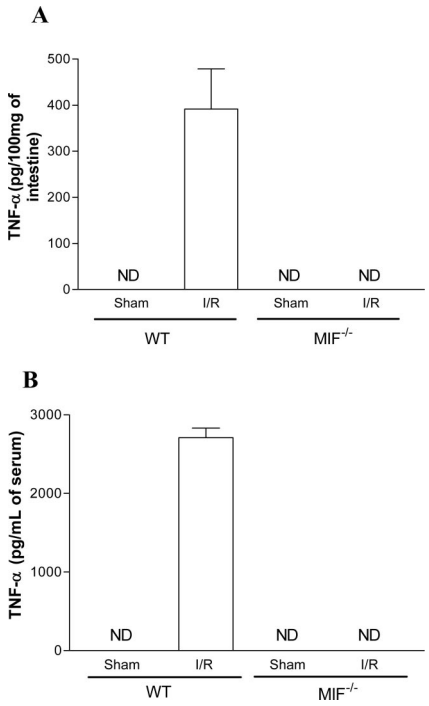

Figure 2. Concentrations of TNF-α in the intestine and serum of WT and MIF$^{-/-}$ mice undergoing intestinal I/R injury. The concentrations of TNF-α in the intestine (**A**) and serum (**B**) of sham-operated mice (Sham) and mice undergoing 60 minutes of ischemia and 60 minutes of reperfusion (I/R) of the superior mesenteric artery were determined by ELISA. Results are shown as pg of TNF-α per ml of serum or per 100 mg of intestine and are mean ± SEM of five animals. ND = not detectable.

reperfused WT and MIF$^{-/-}$ mice (WT: 103 ± 3 to 1069 ± 79; MIF$^{-/-}$: 105 ± 56 to 828 ± 139 pg/100 mg in sham-operated mice and after I/R, respectively).

The histopathological analysis of WT and MIF$^{-/-}$ mice confirmed the biochemical quantification described above. There was no infiltration of leukocytes or other changes in inflammatory parameters in sham-operated WT and MIF$^{-/-}$ mice (Figure 4). In the intestine of reperfused WT mice, the villi were widened with edema and leukocyte infiltration (Figure 4c). In MIF$^{-/-}$ mice, there was a marked reduction in tissue damage, with little edema and diminished erosions on the tips of the villi, despite the intense leukocyte infiltration (Figure 4d). There were also lesions in the lungs. In reperfused WT animals, there was hyperemia, infiltration of leukocytes, and interstitial edema (Figure 4g). In reperfused MIF$^{-/-}$ mice, the interstitial edema and hyperemia were diminished, but the number of leukocytes was similar to those of WT animals (Figure 4h).

Level of Cell Activation as Measured by Chemiluminescence

The level of leukocyte activation was evaluated by measuring the respiratory burst of leukocytes induced by zymosan in a luminol-enhanced chemiluminescence assay. There was no difference in the basal chemiluminescence produced by nonstimulated leukocytes from MIF$^{-/-}$ or WT mice (Figure 5). Addition of zymosan to the leukocytes induced a marked respiratory burst that peaked at ~30 minutes after stimulation. However, the respiratory burst of leukocytes from MIF$^{-/-}$ mice was smaller than that of leukocytes from WT mice (Figure 5). On average, there was an inhibition of 36% of peak respiratory burst.

Lethality after I/R Injury

To confirm the relevance of MIF for the outcome of the systemic inflammatory response triggered by the reperfusion injury, we evaluated the lethality rates of WT and MIF$^{-/-}$ mice submitted to I/R of the superior mesenteric artery. As seen in Figure 6, more than 80% of reperfused WT mice died after 120 minutes of reperfusion. In contrast and despite a similar lethality rate at the first minutes of reperfusion, lethality in MIF$^{-/-}$ was greatly prevented, and more than 20% of the mice were still alive at 120 minutes after reperfusion of ischemic superior mesenteric artery (Figure 6).

Discussion

Several studies have now shown that the release of MIF by cells of the immune system, especially macrophages, may play a relevant role in the physiopathology of certain inflammatory studies.[5,26] A single study has demonstrated that macrophages infiltrating the infarcted myocardium may be a source of MIF.[27] However, the relevance of MIF release was not described in the latter study

Table 1. Changes in Vascular Permeability, Neutrophil Influx, and TNF-α Production in the Lungs of Wild-Type (WT) and MIF-Deficient Mice Undergoing Intestinal Ischemia and Reperfusion Injury

	WT		MIF$^{-/-}$	
	Sham	I/R	Sham	I/R
Evans blue	1.51 (0.32)	4.78 (1.43)*	1.44 (0.33)	2.39 (0.6)†
Neutrophil influx	2250.03 (797.3)	5749.97 (1866.6)*	1914.07 (482.07)	5276.02 (1759.6)*
TNF-α	304.84 (103.28)	1308.24 (75.55)*	344.96 (130.04)	459.8 (90.87)†

All parameters were measured in sham-operated mice (Sham) and after 60 minutes of ischemia followed by 60 minutes of reperfusion (I/R) of the superior mesenteric artery. Changes in vascular permeability were assessed as the amount of Evans blue that extravasated into the tissue and is expressed as μg of Evans blue per 100 mg of tissue. The concentrations of TNF-α are shown as pg of TNF-α per 100 mg of tissue and neutrophil influx is given as relative units as shown in the Materials and Methods section. Results are mean ± SEM of five animals in each group. All experiments were repeated twice.

*$P < 0.01$ when compared to sham-operated mice.

†$P < 0.01$ when compared to WT undergoing I/R.

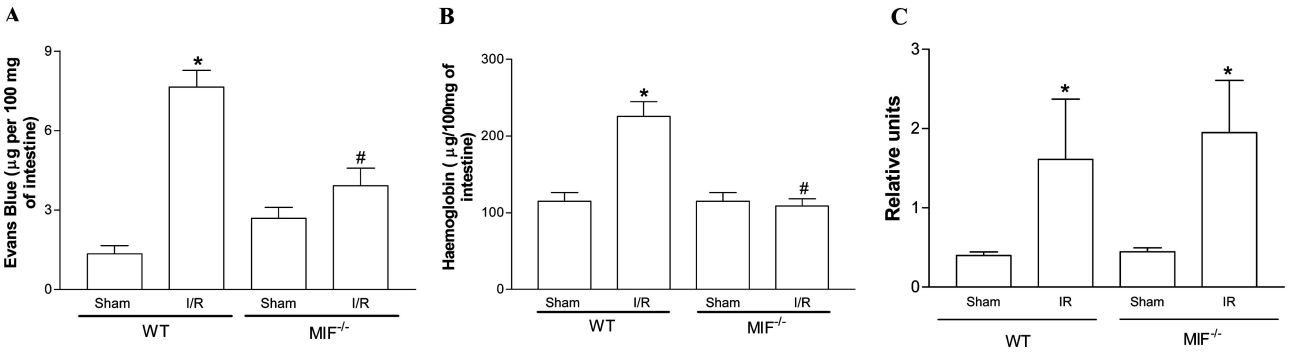

Figure 3. Changes in vascular permeability (**A**), hemorrhage (**B**), and neutrophil infiltration (**C**) in the intestine of WT and MIF$^{-/-}$ mice undergoing intestinal ischemia and reperfusion injury. All parameters were measured in sham-operated mice (Sham) and after 60 minutes of ischemia followed by 60 minutes of reperfusion (I/R) of the superior mesenteric artery. **A:** Changes in vascular permeability were assessed as the amount of Evans blue that extravasated into the tissue and is expressed as μg of Evans blue per 100 mg of tissue. Hemorrhage was evaluated as the amount of hemoglobin in 100 mg of tissue (**B**) and neutrophil influx as the tissue myeloperoxidase content (**C**). Neutrophil influx is given as relative units as shown in the Materials and Methods section. Results are mean ± SEM of five animals in each group. $^{*}P < 0.01$ when compared to Sham-operated mice and $^{\#}P < 0.01$ when compared to WT undergoing I/R. All experiments were repeated twice.

or in other forms of reperfusion-associated injury and lethality. In the current study, we evaluated the role of MIF in a model of I/R of the superior mesenteric artery in mice. Our results indicate that the cytokine MIF drives TNF-α production and plays a relevant role in mediating reperfusion-induced inflammatory injury and lethality in mice.

In mice submitted to reperfusion of the ischemic superior mesenteric artery, there is an acute inflammatory response that is accompanied by significant tissue injury and lethality of animals.[19,20,28] Studies from our and other laboratories have clearly shown that the inflammatory response, characterized by marked edema formation and neutrophil influx, is necessary for tissue injury and lethality to occur.[29,30] In MIF$^{-/-}$ mice, there was a clear reduction of tissue injury, as assessed by the diminished levels of hemoglobin in the intestine and histopathology of the lungs and intestine. Moreover, prevention of local and remote injury was accompanied by inhibition of lethality from 80% in WT to 20% in MIF$^{-/-}$ mice. The protection induced by absence of MIF in this model of reperfusion is consistent with the active proinflammatory role of this cytokine in other models of tissue injury[11,31] but is the first demonstration that MIF is relevant during reperfusion-induced injury and lethality.

In MIF$^{-/-}$ mice, there was a suppression of edema formation but the accumulation of neutrophils was similar to those of WT mice, as assessed by evaluating tissue myeloperoxidase content and histopathology. The inhibition of edema formation is consistent with other studies evaluating this parameter in different models of inflammation.[26,32] However, a few studies have demonstrated that blockade of MIF with antibodies or experiments in

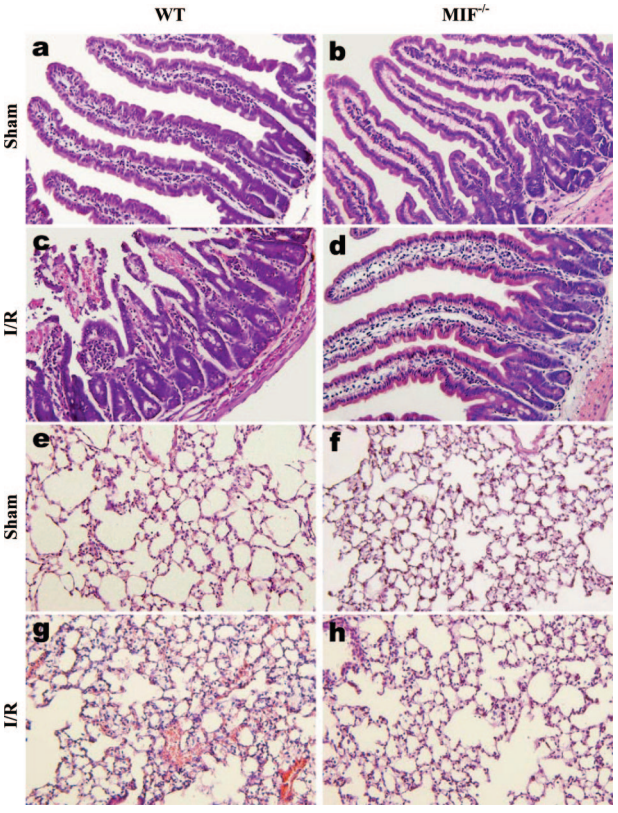

Figure 4. Histopathological analysis of the intestines (**a–d**) and lungs (**e–h**) of WT and MIF$^{-/-}$ mice undergoing intestinal I/R injury. Tissues were obtained from sham-operated mice (Sham) and after 60 minutes of ischemia followed by 60 minutes of reperfusion (I/R) of the superior mesenteric artery. Sections were stained with H&E. Original magnifications, ×100.

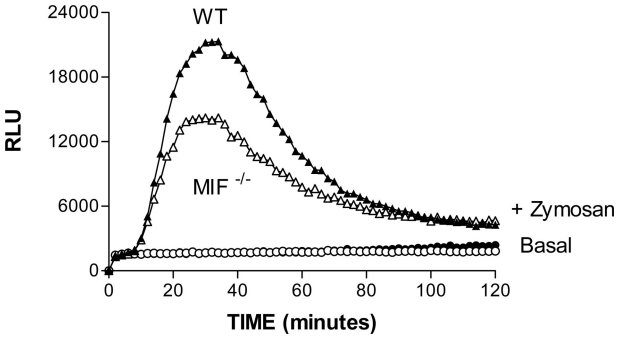

Figure 5. Time course of luminol-enhanced chemiluminescence in leukocytes obtained from lungs of WT and MIF$^{-/-}$ mice undergoing intestinal I/R injury. Leukocytes pooled from lungs of five mice after 60 minutes of ischemia followed by 60 minutes of reperfusion (I/R) of the superior mesenteric artery. Leukocytes (3×10^5 cells/well) were left unstimulated (basal) or were stimulated with zymosan (20 particles/cell). The relative luminescence units (RLUs) were assayed in triplicate and results are representative of two different experiments.

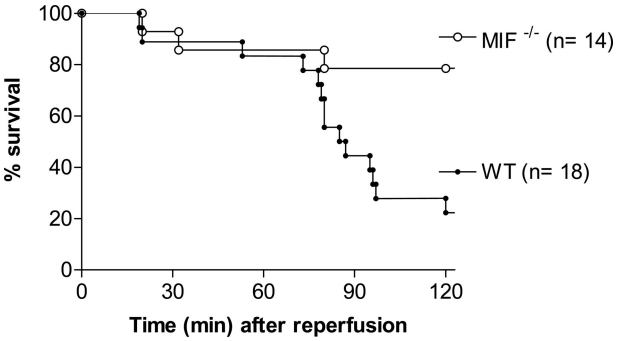

Figure 6. Survival curves of WT and MIF$^{-/-}$ mice undergoing intestinal I/R injury. Mice were anesthetized and submitted to 60 minutes of ischemia of the superior mesenteric artery. The vessel was reperfused, and survival was monitored as indicated. This graph represents one of two similar experiments, and there was a significant difference ($P < 0.01$) between WT and MIF$^{-/-}$ mice.

MIF$^{-/-}$ mice prevented the influx of leukocytes into tissue.[33,34] A possible mechanism to explain the inhibitory effects of MIF on leukocyte accumulation is the ability of MIF to modulate the expression of adhesion molecule expression necessary for leukocyte recruitment.[35] It was somewhat surprising to note that neutrophil accumulation was similar in WT and MIF$^{-/-}$ mice submitted to I/R injury. Consistently with the latter observation, we observed that the number of circulating neutrophils (data not shown) and the levels of KC were similar in both WT and MIF$^{-/-}$ mice. Indeed, KC and other interleukin-8-like CXC chemokines are produced during intestinal I/R injury and together with their CXCR2 receptor mediate the influx of neutrophil into ischemic tissues.[36] Blockade of CXCR2 or antibody against the cytokines prevent not only neutrophil influx but also tissue injury and lethality,[20,36,37] demonstrating that neutrophils are essential for I/R-associated injury and lethality. Because neutrophil recruitment was similar in WT and MIF$^{-/-}$ mice and neutrophils are essential for injury to occur, one possibility to explain our results would be that the activation of neutrophils is impaired in MIF$^{-/-}$ mice submitted to reperfusion injury. This possibility was strengthened by our observation that luminol-enhanced chemiluminescence induced by zymosan, a measure of respiratory burst, was diminished in cells obtained from MIF$^{-/-}$ mice submitted to I/R injury. The latter result could be explained by a direct action of MIF on the leukocyte or the impaired production of cytokines, such as TNF-α, capable of priming neutrophils in an autocrine/paracrine manner. Indeed, at least one study has shown that MIF may directly activate or modify the activation of neutrophils.[33]

One alternative explanation for the lack of difference in neutrophil influx would be a differential rate of apoptosis between WT and MIF$^{-/-}$ mice. That is, if apoptosis were slower or less intense in MIF$^{-/-}$ mice, this could lead to similar accumulation of cells at the end of the experiment even though recruitment was different. Previous studies have shown that MIF can indeed delay polymorphonuclear cell apoptosis.[38] However, we failed to find an increased level of apoptosis over baseline, as assessed by terminal dUTP nick-end labeling or fluorescence-activated cell sorting (data not shown), in leukocytes obtained from tissue of reperfused animals. It is worth noting that reperfusion injury is very severe in our system, and leukocyte accumulation is measured within a very short time frame (60 minutes), probably reflecting our inability to show an increase in the number of apoptotic cells.

The cytokine TNF-α is expressed during reperfusion of ischemic vascular territories, and several studies have shown that TNF-α plays a relevant role for reperfusion-induced tissue injury and lethality.[19,20] Because the rapid release of MIF may be involved in the production of TNF-α in certain experimental models,[5,10,23] we investigated the levels of TNF-α in WT and MIF$^{-/-}$ mice. Our results showed that MIF$^{-/-}$ mice had greatly diminished quantities of TNF-α in intestine, lungs, and serum after reperfusion injury. The diminished concentrations of TNF-α in MIF$^{-/-}$ mice are consistent with an inhibition of neutrophil activation and diminished reperfusion-associated injury and lethality observed in these animals. Indeed, TNF-α may activate neutrophils directly[39] or prime neutrophil activation induced by other mediators.[40]

Altogether, our results demonstrate an essential role for MIF in mediating the local, remote, and systemic changes induced by reperfusion of the ischemic superior mesenteric artery. In this system, MIF does not appear to be relevant for the recruitment of neutrophils but is essential for TNF-α release, suggesting that the latter mechanism may underlie the inflammatory functions of MIF in the system. It is possible that MIF-based strategies may be useful adjuncts in the therapy of patients undergoing reperfusion injury.

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